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Analysis of the bioavailability of Cr(III) and Cr(VI) based on the determination of chromium in *Mentha piperita* by graphite furnace atomic absorption spectrometry

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Abstract: *Mentha piperita* L. (*Lamiaceae*) was cultivated under the controlled laboratory conditions in the presence of varying levels of trivalent and hexavalent chromium in order to determine its capacity to control chromium uptake and its tolerance limit. The plants were grown in pots at 25 °C with controlled soil moisture (about 80 % of the water retention capacity). The soil was treated with increasing concentrations of $Cr(NO_3)_3$ (40, 80, 120, and 200 mg kg^{-1}) and $K_2Cr_2O_7$ (2.5, 5, 10, and 15 mg kg^{-1}). A control group of plants was grown without the addition of chromium to the soil. For each concentration, three acidity levels were tested: natural, one pH unit below and one above the natural acidity of the soil (pH_2 6, pH_1 5 and pH_3 7). The plant samples were digested according to the standard procedure and chromium content was determined by GFAAS. For all plants, the transportation index was calculated and the results (expressed in mg kg^{-1}) at pH_1, pH_2 and pH_3, respectively, were: 0.21–0.80, 0.06–1.06 and 0.04–0.52. The recoveries were good (72.73–115.3 %) as evidenced by the analysis of certified reference materials (NIST SRM 8433 – Corn Bran and NIST SRM 1547 – Peach Leaves). The mobility of chromium through the plants tissues is discussed in regard to its competition with iron and manganese for transport binding sites; hence Mn and Fe were also determined.

Keywords: chromium; GFAAS; uptake; translocation; toxicity; *Mentha piperita*.

INTRODUCTION

Environmental protection is of global importance and could be generally discussed in terms of anthropological and natural sources of pollution. Identification of sources followed with a systematic analytical approach is an acceptable mode

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of action, from which a real contribution could be expected. When chromium is concerned, anthropogenic sources are dominant and significantly contribute to environmental pollution.

The chemistry of chromium is very complex and its solubility and mobility in soils and its bioavailability strongly depend on the various oxidation states of this metal (from 0 to +6), its concentration, soil acidity, redox potential and salinity. Depending on its oxidation state and concentration, chromium acts as toxic or as an essential element for animals and humans. The two most common chromium species are Cr(III) and Cr(VI) in its anionic forms as chromate, dichromate and hydrochromate ions. Cr(III) is essential for animals and humans at low concentrations. It is very stabile in soils, but commonly well immobilized on iron and manganese oxides and hydroxides or complexed to organic matter. The toxicity of Cr(VI) depends strongly on its concentration in the soil as its uptake mechanism is based mainly on passive diffusion. It is still questionable if the trivalent chromium is essential for plants, but at high concentrations it definitely inhibits plants growth while a certain decrease in the activity of the immune system was observed in humans.[1] Toxicity is connected with solubility and, from this point of view, ammonium and alkaline metal salts of chromic acid, as very soluble species in the soil, should be recognized These species are toxic for plants, animals and humans. Symptoms of Cr(VI) toxicity in humans include skin irritation, gastric distress and liver damage. In addition some species formed in the reduction process from hexavalent to trivalent chromium contribute to the cytotoxicity, genotoxicity and carcinogenicity of Cr(VI). The uptake mechanism of this chromium species is active transport, probably correlated with the sulfate transport system of plasma membrane.[3,4]

In plants, chromium reduces the content of proteins, inhibits the activity of enzymes activity, decreases plant growth, and causes chlorosis and necrosis.[5] Barcelo *et al.* found a high correlation between the concentration of chlorophyll pigments and iron uptake in chromium stressed plants.[6] Many authors have reported about the negative effects of chromium on iron and manganese absorption.[2,7–10] Chromium is known to compete with both these metals for transport binding sites.

The relationships between chromium concentrations and contamination of the soil and its concentration in plants are extremely important, not only from the aspect of its influence on plant growth and capacity of plant accumulation, but also for potential toxicity for humans. Chromium accumulated in medical plants enters the food chain and, in this way, increases the potential health risk.

The main objectives of this study were to analyze the bioavailability of two chromium species (Cr^{3+} and the chromate) in contaminated soil and its potential uptake by *Mentha piperita*. Additionally, the influence of the two species on iron and manganese absorption was also investigated. The importance of soil acidity

to chromium mobilization and accumulation was recognized and a systematic empirical approach in the experimental design was developed. For this purpose, *M. piperita* was cultivated under controlled laboratory conditions, on the soils at different acidity levels, treated with Cr^{3+} and $Cr_2O_7^{2-}$. The conducted study was also aimed at elucidating the accumulation and translocation of chromium from the root to the upper plants parts. The obtained results were expected to enable the determination of the tolerance levels for *M. piperita* and also the chromium concentrations when the symptoms of toxicity become visible.

EXPERIMENTAL

Solutions and reagents

All employed reagents were of analytical grade. Single and multi-element calibrant solutions were prepared from 1.0 g L^{-1} *p.a.* stock solutions (Merck, Germany). For the microwave-assisted acid digestion, HNO_3 65 %, *p.a.* (Baker, Holland) and H_2O_2 30 %, *p.a.* (Merck, Germany) were used. The chromium solutions were prepared from reagent grade salts, $Cr(NO_3)_3$ (Fluka, Switzerland) and $K_2Cr_2O_7$ (Merck, Germany). All solutions were prepared with deionized water (Milli-Q system: resistivity 18.2 MΩ cm, TOC < 10 μg L^{-1}).

Soil characterization and preparation procedure

Soil was collected from the plantation of the Institute "Dr Josif Pančić" in Pančevo. The sampling procedure, as well as the measurement of acidity (model HI 9017 pH Meter, Hanna Instruments) were realized according to a procedure presented elsewhere.[11] The measured natural acidity was pH 5.98. The analyzed soil was poorly calcareous. Carbonates were determined by the Scheibler test* and calculated as $CaCO_3$, 0.16 %. The humus content (2.67 %) was determined by the method described by Tjurin and modified by Simakov[12] and total organic carbon (*TOC*) (2.97 %) by an official procedure for waste, sludge and sediments.**

Additionally, the retention water capacity (*RWC*) and the amount of acid (0.10 M HCl) and alkaline (0.10 M NaOH) required to adjust the pH by ±1, using the corresponding buffer curves for soil, were determined.

About 1.5 kg of air-dried soil was weighed and kept in plastic pots with a hole in the bottom to enable drainage. Then, *M. piperita* was planted in each pot.

Plant cultivation and treatment

The rhizomes of *M. piperita L. (Lamiaceae)* were sampled in a field of the Institute for Medical Plant Research "Dr Josif Pančić" in Pančevo. The plants were cultivated at a temperature of about 25 °C under laboratory conditions and natural light. The soil moisture was maintained at about 80 % of the retention water capacity (*RWC*). When the plant specimens had developed and were about 10 cm in height, the soil was treated with increasing concentrations of $Cr(NO)_3$ (40, 80, 120, and 200 mg kg^{-1}) and $K_2Cr_2O_7$ (2.5, 5, 10, and 15 mg kg^{-1}). These concentration levels were selected to obtain two levels below and two above the maximal allowable concentration (*MAC*) for Cr(III) and additionally two levels below and two above the toxic level of 5 mg kg^{-1} for plants.[5,8] Control plants were grown in soil without the

* NEN5757, 1991. The Scheibler test determination of carbonate concentration in soils. Volumetric method (in Dutch). Normalisatie Instituut, Delft, The Netherlands.

** Draft European Standard procedure (chemical analyses – determination of total organic carbon (*TOC*) in waste, sludges and sediments, Method B (direct method), document type: Draft European Standard, STD Version 2.1a (20020903), 2004.

addition of chromium. For each concentration level, sets of three additional samples were prepared at three acidity levels: natural, one pH-unit below and one above the natural acidity of the soil using buffer curves obtained for acid (0.10 M HCl) and alkaline (0.10 M NaOH) conditions.[13] With a natural acidity of pH 5.98, pH 5.00 and pH 7.00 were selected for the experiments.

Sample preparation

After sampling, the plants were separated into herbal parts and roots. Each part was washed, first with tap and then with distilled water and dried. Further sample preparation was applied as described elsewhere.[11,13] For the microwave-assisted acid digestion of the plant samples, the SW-846 EPA Method 3052 was applied.[14] A mixture of an accurately weighed amount of plant sample (*ca*., 0.4 g), 12 ml of 65 % HNO_3 and 4 ml of 30 % H_2O_2, after waiting 10 min for the first vigorous chemical reaction to subside, were digested according to the temperature program presented elsewhere.[11,13] After cooling, the samples were filtered through 0.45 µm Millipore filters. The solutions were quantitatively transferred into 50-ml volumetric flasks and diluted to volume with deionized water.

Instrumental and operating conditions

The determinations of iron and manganese were performed on a Perkin–Elmer Model 5000 atomic absorption spectrophotometer, under optimized measurement conditions using suitable hollow cathode lamps. The signals were measured with background correction (deuterium lamp) at the optimal flame (A–Ac) height.[11,13]

The determination of Cr in all soil and plants extracts was performed using a Perkin–Elmer model 5000 atomic absorption spectrophotometer with a graphite furnace HGA 400 Automatic Burner Control, with pyrollytic graphite tubes and temperature programs presented elsewhere.[11,13]

External calibration was applied for the determination of the metals and the corresponding regression equations were calculated as follows: for Cr, $y = 0.0033x + 0.062$; for Fe, $y = = 0.0437x + 0.0001$ and for Mn, $y = 0.1567x + 0.0016$. The correlation coefficients were in the r^2 range 0.989–0.999.

The good reproducibility of results was proved by the relative standard deviations (*RSD*) values of up to 2 %. All the results presented in Table I represent the average of five sample measurements. The results were rounded up to the last figure associated with random error. The significance level, α, was 0.05. Data analysis was realized using Office Excel 2007.

Analysis of the certified reference materials

The accuracy of the methods applied for determination of Cr, Mn and Fe, after microwave-assisted acid digestion of plants and soil samples, was checked by analysis of SRMs and the obtained results are presented in Table I.

TABLE I. Analysis of the certified references materials

Element	NIST SRM 8433 – Corn Bran			NIST SRM 1547 – Peach Leaves		
	Found mg kg^{-1}	Certified value mg kg^{-1}	Recovery %	Found µg kg^{-1}	Certified value µg kg^{-1}	Recovery %
Cr	0.08	0.11	72.73	890	1000	89.0
Mn	2.10±0.42	2.55±0.29	82.35	113±8	98±3	115.3
Fe	15.7±1.7	14.8±1.8	106.08	199±12	218±14	91.28

Transportation index

The transportation index (*TI*, average Cr content in the herbal plant part, mg kg^{-1}/Cr content in the roots, mg kg^{-1}) was calculated to evaluate the ability of the plants to translocate metal from roots to the upper part of the plant.[13,15–17]

RESULTS AND DISCUSSION

Visible symptoms of chromium toxicity

Decreases in growth and symptoms of toxicity were observed after 18 days in plants cultivated on the soils treated with 120 and 200 mg kg^{-1} Cr(III) and 10 and 15 mg kg^{-1} Cr(VI) under all acidity conditions. After an additional 20 days, the leaves became brownish-red and a reduction of the number and size of leaves was also noticed. In the next 8 days, small necrotic areas were registered on the plants cultivated on the soil treated with 15 mg kg^{-1} Cr(VI). After an additional 5 days, the plant species became dry. In all plants, a poorly developed root system was found.

These observations are in accordance with some reported data.[5,7,9] With concentrations higher than 5 mg kg^{-1}, the hexavalent chromium damaged the root membranes because of its high oxidation power and caused changes in metabolic processes, such as a decrease in chlorophyll synthesis, chlorosis and inhibition of root and plant growth.[5,8,10]

Metal analysis

The concentrations of metals (Cr, Fe and Mn) in *M. piperita* plant samples cultivated on untreated and treated soils are presented in Figs. 1a–1c and in Tables II and III.

The obtained results (Figs. 1a–1c) show that uptake of chromium by *M. piperita* as relatively low from all soils treated with Cr(III) and Cr(VI), at all investigated pH values. This could be explained by the very good immobilization of chromium in the soil by iron and manganese hydroxides and oxides.[8,13] The total chromium content in the herbal part of the plants grown on soil with the native pH of ranged 0.137 to 2.425 mg kg^{-1} for Cr(III) and from 0.334 to 5.025 mg kg^{-1} for Cr(VI), and generally increased with increasing concentration of this metals in the soil (Fig. 1b).

The total Cr content in the roots of *M. piperita* at the native pH value of the soil was from 1.780 to 4.715 mg kg^{-1} for Cr(III) and from 2.201 to 4.304 mg kg^{-1} for Cr(VI). The analysis of *M. piperita* showed several times higher concentrations of chromium in the roots than in the upper plant parts cultivated on soil treated with Cr(III) or Cr(VI), with exception of plants grown on the 10 mg kg^{-1} hexavalent chromium treated soil (Figs. 1a–1c). Zayed and Terry came to a similar conclusion about the ability of plants to hold more than 90 % of chromium in their roots.[8] The obtained results showed a better capacity of *M. piperita* to accumulate Cr in the roots than in the upper plant parts.

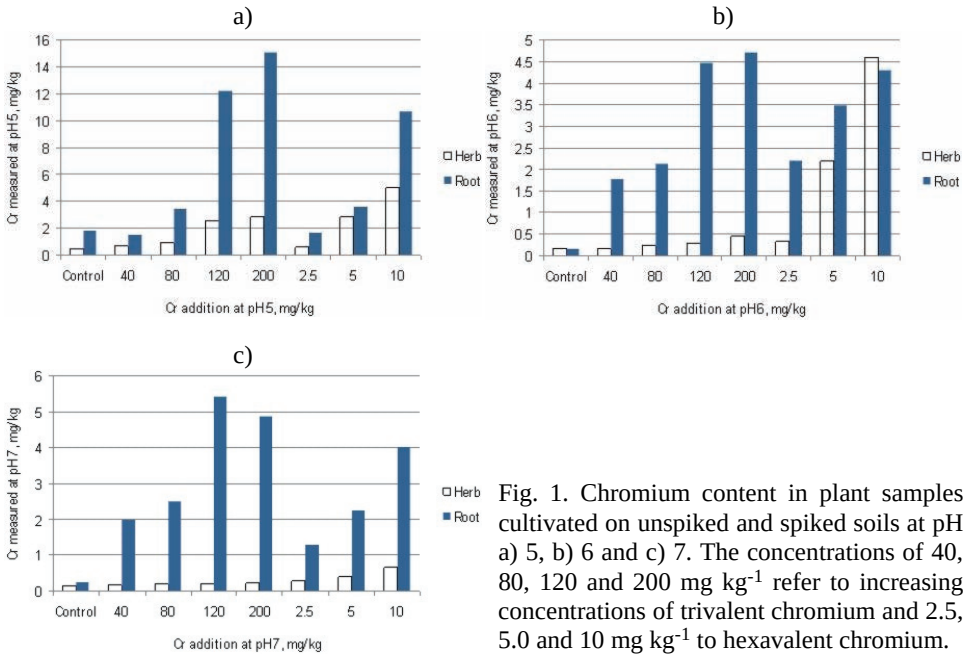

Fig. 1. Chromium content in plant samples cultivated on unspiked and spiked soils at pH a) 5, b) 6 and c) 7. The concentrations of 40, 80, 120 and 200 mg kg^{-1} refer to increasing concentrations of trivalent chromium and 2.5, 5.0 and 10 mg kg^{-1} to hexavalent chromium.

TABLE II. Iron and manganese contents in the herbal part of *M. piperita* cultivated on treated and untreated soils at three acidity levels, expressed in mg kg^{-1}

Sample	pH					
	Mn			Fe		
	6	5	7	6	5	7
$H_{(Control)}$	46.17	44.23	47.39	322.5	354.3	376.3
$H_{(40)}$[a]	44.64	45.32	30.95	320.2	355.6	383.0
$H_{(80)}$	42.68	69.00	29.48	297.4	320.9	356.4
$H_{(120)}$	49.24	68.82	27.57	297.4	318.2	349.9
$H_{(200)}$	52.96	41.78	32.75	263.0	367.3	333.3
$H_{(2.5)}$	42.80	43.88	21.42	345.6	356.4	367.7
$H_{(5)}$	53.15	36.14	34.59	320.0	378.2	358.0
$H_{(10)}$	61.25	51.89	30.05	408.6	368.4	374.3

[a]H(40)–H(200) and H(2.5)–H(10) – herbal (H) samples from the plants cultivated on soils spiked with increasing concentrations of trivalent chromium (40, 80, 120 and 200 mg kg^{-1}) and hexavalent chromium (2.5, 5.0 and 10 mg kg^{-1})

It is important to stress that plants cultivated on untreated soil at pH 6 had almost the same concentration in the roots and in the herbal part (Fig. 1b), but with increasing concentration of chromium in the soil, the transfer efficiency decreased which is in accordance with reported data.[8–110,15,18]

The calculated transportation indexes additionally supported the found poor translocation of chromium from the roots to the upper plant parts (Table IV). The

calculated values for *TI* were five times lower in the plants cultivated on the soil treated with Cr(III) than those cultivated on soil treated with Cr(VI). Higher values for *TI* were calculated for the plants cultivated on the soils treated with concentrations of 5 and 10 mg Cr(IV) kg^{-1}, when the symptoms of oxidative stress were observed. These results support a higher mobility of Cr(VI) through the plant tissues.

TABLE III. Iron and manganese contents the in root part of *M. piperita* cultivated on treated and untreated soils on three acidity levels, expressed in mg kg^{-1}

Sample	pH					
	Mn			Fe		
	6	5	7	6	5	7
$R_{(Control)}$	95.41	76.10	85.24	3363.7	4621.8	3778.4
$R_{(40)}$[a]	141.4	49.39	55.18	4588.0	4752.1	3834.6
$R_{(80)}$	118.0	71.14	55.19	3947.2	4509.4	3698.4
$R_{(120)}$	85.67	100.41	35.65	3329.4	4655.9	3714.6
$R_{(200)}$	75.22	59.84	48.86	2780.2	4312.3	3708.2
$R_{(2.5)}$	89.64	78.61	36.72	3457.9	4218.4	4017.1
$R_{(5)}$	44.64	49.55	47.55	4683.3	4682.6	3896.2
$R_{(10)}$	78.26	93.61	42.15	3785.9	4278.2	4103.5

[a]R(40)–R(200) and R(2.5)–R(10) – root (R) samples from the plants cultivated on the soils spiked with increasing concentrations of trivalent chromium (40, 80, 120 and 200 mg kg^{-1}) and hexavalent chromium (2.5, 5.0 and 10 mg kg^{-1})

TABLE IV. Transportation index (*TI*) for chromium in plants cultivated on untreated and spiked soils with chromium

Chromium species	Content, mg kg^{-1}	*TI*		
		pH		
		6	5	7
Control	0	0.99	0.23	0.52
Cr(III)	40	0.09	0.45	0.08
Cr(III)	80	0.12	0.27	0.07
Cr(III)	120	0.06	0.21	0.04
Cr(III)	200	0.10	0.19	0.05
Cr(IV)	2.5	0.15	0.34	0.23
Cr(IV)	5.0	0.63	0.80	0.18
Cr(IV)	10	1.06	0.47	0.17

Chromium uptake and transport are dependent on its chemical form and the hexavalent species is more mobile than the trivalent one.[1,2,17,18] Torrestday *et al.* reported that accumulation of chromium in the upper plant parts is 12 to 18 times higher for hexavalent than for trivalent chromium.[1] These two species have an independent mechanisms of uptake.[17] The uptake of Cr(III) is passive diffusion and this ion interacts with cell walls through cation-exchange sites.[2,18] Absorption of hexavalent chromium depends on metabolic energy of plants.[2] Cr(VI)

moves more easily from the roots to upper plants tissues because of the absorption, especially of its $Cr_2O_4^{2-}$ form, as an active process, probably correlates with the sulfate transport system located in the plasma membrane.[3,4,19] It is important to stress that despite of the easy way of Cr(VI) absorption, the fact that the *TI* values were not very high is probably due to the ability of the plant to reduce Cr(VI) to the less toxic Cr(III) form at the roots level.[1,10,20] The reduction could be catalyzed by Fe-3-reductase enzymes.[8] This is probably one of the natural plant mechanisms enabling tolerance to high concentrations of chromium.

Bearing in mind possible slight changes of acidity in the environment, it is important to stress that the *TI* values of Cr(III) showed no changes with increasing pH from natural 6 to 7 (Table IV) at all investigated concentration levels in the soil.

However, an increasing tendency of *TI* was noticed at pH 5 (Table IV), which suggests a changed ability of roots and herbal parts to accumulate chromium. From the obtained results, a higher mobility of chromium in the soil at lower acidity could be assumed.[13,21] This is in accordance to other findings that oxidation of Cr(III) to much more mobile Cr(VI) in soil is increased at pH 5.[8] This process is generally very slow under conditions of natural normal acidity.

Being a non-essential element and also toxic for plants, there is no specific mechanism for chromium transport through plants and this metal is known to compete with iron and manganese for transport binding sites.[10] The concentration of manganese generally decreased with increasing Cr levels.[10] The present results (Figs. 1a–1c, Tables II and III) do not show significant correlations between the Cr(III) and Cr(VI) contents in the roots and herbal parts and the concentrations of manganese at the investigated acidity levels. Dube *et al*. came to a similar conclusion.[7] However, comparing the Cr(III) concentrations with the concentrations of Fe (Table II), a negative correlation (R^2 = 0.9851) was noticed in the herbal part of *M. piperita* (Fig. 2). Torrestday *et al*. reported a decrease of the iron concentration in leaf tissue in response to chromium toxicity.[1]

The similarity of the ionic radii of Cr^{3+} and Fe^{3+} species may enable the replacement of iron with chromium in the heme proteins.[3,8,9] It has been found a high correlation between chlorophyll pigments and Fe uptake in chromium stressed plants was found.[6] Despite the similar chemical characteristics of Fe(III) and Cr(III), much higher concentrations of iron (263.0 to 408.6 mg kg^{-1}) than chromium (0.161 to 2.825 mg kg^{-1}) were present in the investigated samples (Table II; Figs. 1a–1c).There is a reasonable explanation for the high immobility of chromium in soil–plant systems: no reduction of Cr(III) to Cr(II) is observed under natural conditions while a relatively easy reduction of Fe(III) to Fe(II) can occur.[5,8,13] A comparison with the control plant samples showed that the concentrations of Fe in the roots was not significantly affected.

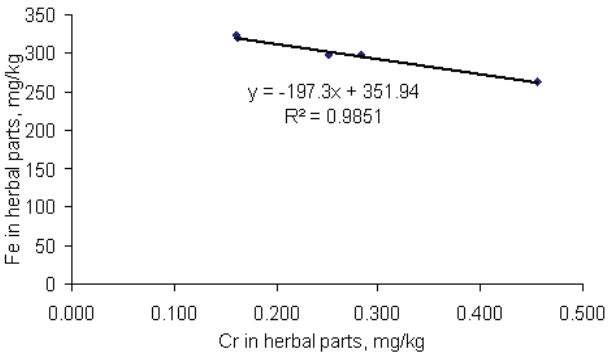

Fig. 2. Correlation graphs of the chromium and iron content in the herbal parts of *M. piperita*.

CONCLUSIONS

In this study, the capacity of the medicinal plant *Mentha piperita* (*L. Lamiaceae*) to control chromium uptake and its tolerance limit on soils treated with different chromium concentrations under conditions of three different pH levels were analyzed.

In order to realize this, *M. piperita* was cultivated under controlled laboratory conditions on soils treated with different concentrations of trivalent and hexavalent chromium.

The obtained results showed relatively low uptakes of chromium by *M. piperita* for all types of soils, treated with both trivalent and hexavalent chromium, at all the investigated pH values, probably due to the good immobilization of chromium in the soil matrix. The total chromium content in the plant, in general, increased with increasing soil concentration of the metal. These concentrations were several times higher in the roots than in the herbal parts of plant which indicated a very good root holding capacity (around 90 %). Exceptions were noticed with plants grown on the more acidic soils (pH 5) because of a poorer holding capacity of roots, probably as a result of damage to the membranes of the root cells.

The translocation indexes calculated for Cr(III)-contaminated plants showed low mobility of this chromium species, which is in correlation with its passive mechanism of uptake. In addition, the negative correlation between the Cr(III) and the Fe concentrations in the herbal plant parts confirms their competition for the transport binding sites.

The obtained results for Cr(VI) showed a very high mobility of hexavalent chromium through the plants tissues, which is in correlation with active uptake mechanism of this chromium species.

The medicinal plant *M. piperita* L. *(Lamiaceae*) analyzed in this work had a large capacity for binding chromium in the root system, which is a very important protection mechanism of the plant from the toxic action of this metal.

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И З В О Д

АНАЛИЗА БИОДОСТУПНОСТИ Cr(III) И Cr(VI) ЗАСНОВАНА НА ОДРЕЂИВАЊУ ХРОМА У БИЉЦИ *Mentha piperita* МЕТОДОМ АТОМСКЕ АПСОРПЦИОНЕ СПЕКТРОМЕТРИЈЕ СА ГРАФИТНОМ КИВЕТОМ

СВЕТЛАНА ЂОГО[1], СЛАВИЦА РАЖИЋ[1], ДРАГАН МАНОЈЛОВИЋ[2] и ЛАТИНКА СЛАВКОВИЋ[3]

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Циљ овог рада је био анализа лимита толеранције за усвајање Cr(III) и Cr(VI) из загађеног земљишта и одређивање капацитета медицинске биљке *Mentha piperita* (*L. Lamiaceae*) ради контроле уноса овог метала. Биљке су узгајане у пластичним посудама, при собној температури од 25 °C и одржаваној влажности земљишта на око 80 % ретенционог воденог капацитета. Земљиште на коме су биљке узгајане контаминирано је раствором Cr(III)-нитрата (40, 80, 120, и 200 mg kg^{-1}) и $K_2Cr_2O_7$ (2,5; 5; 10; и 15 mg kg^{-1}). Контролна група биљака је расла под истим условима без додавања хрома. За сваку концентрацију хрома у земљишту припремане су серије узорака на три нивоа киселости: природни и за по једну јединицу киселији и базнији (pH_2 6, pH_1 5 and pH_3 7). Узорци биљака су припремани за анализу према стандардној процедури и хром је одређиван методом атомске апсорпционе спектрометрије са графитном кивету, GFAAS. За узорке на сва три нивоа киселости израчунат је транспортациони индекс и добијене вредности су у следећим интервалима: 0,21–0,80; 0,06–1,06; 0,04––0,52. Тачност методе (72,73–115,3 %) је потврђена анализом хрома у узорцима стандардних референтних материјала (NIST SRM 8433 – Corn Bran and NIST SRM 1547 – Peach Leaves). Мобилност хрома из кореновог система у надземни део биљке је дискутована и са аспекта компетиције са гвожђем и манганом за иста транспортна места, па су у том смислу у узорцима одређене и концентрације ових метала.

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